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Reduction of Growth Rate as the Major Process in the Miniaturization of the Sand Dollar *Sinaechinocyamus mai*

CHANG-PO CHEN AND CHI-MING CHAO

Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, Republic of China

Abstract. *Sinaechinocyamus mai* is an extremely small sand dollar, the maximum size being 10.9 mm. It has been suggested that *Sinaechinocyamus* is a miniaturized progenetic sand dollar that closely resembles the juveniles of *Scaphechinus*. In this study, we investigated the mechanisms responsible for the miniaturization. Our analysis of population dynamics, maturity, and annual reproductive cycles suggests that the growth rates of *S. mai* are about 19% the growth rates of *Scaphechinus mirabilis*, which reaches a maximum size of 88 mm. The developmental stages of oral and aboral surfaces were defined on the basis of the number of discontinuous interambulacral plates and the number of tube-foot porepairs, respectively. The patterns of the oral and aboral surfaces of the two species were compared, both at original size and after the *Scaphechinus mirabilis* pattern had been reduced to a size proportional to that of *S. mai* (*i.e.*, to 19% original). On the oral surface, the patterns were different at the original sizes, but similar when the proportional sizes were compared; this indicates that the development of the oral plates is age-dependent in *S. mai*. On the aboral surface, the patterns were similar at the original sizes, but different in the proportional comparison, indicating that the development of the aboral plates is size-dependent in *S. mai*. *S. mai* becomes sexually mature at the age of 2 years, and *Scaphechinus mirabilis* matures probably at about the same age. Our data suggest that the reduction of growth rate (neoteny) is a more important mechanism of miniaturization in *S. mai* than is precocious cessation (progenesis).

Introduction

Miniaturization is the evolution of small adult body size and is a widespread phenomenon in animals (for review, see Hanken and Wake, 1993). The developmental mechanism that mediates evolutionary change in body size and form has been addressed in a number of studies in terms of heterochrony (McKinney and McNamara, 1991). Changes in onset timing, offset timing, and growth rate in the entire individual (global heterochrony) or only in different characteristics but not throughout the individual (dissociated heterochrony), or even in opposite processes (mosaic heterochrony) have diversified body size and form (Skelton, 1993).

The direct mechanisms that result in miniature body size are the precocious cessation of growth, known as progenesis, and the reduction of growth rate, known as neoteny. Precocious cessation of growth means that the ancestral patterns of growth in mass and allometry are unchanged, but the duration of growth is truncated. Thus, lifespan is reduced by truncating growth and development, the reproductive system becomes mature precociously, and all developmental stages remain in the juvenile form of the ancestor. Reduction of growth rate means that the ancestral rate of growth in mass is reduced, but the pattern of allometry and duration of growth remains unchanged. Thus, all developmental and maturation rates remain consistent with those of the ancestor. Although the morphological characteristics are present at the expected age, they appear on a smaller scale as a result of the reduction in growth rate. The descendant is thus a microcosm of its ancestor. Indeed, different characteristics may have different heterochronic processes—the mechanism of miniaturization may not be simple at all.

Sinaechinocyamus mai, an endemic species in Taiwan, is an extremely small sand dollar, the maximum size being 10.9 mm in over 2000 individuals collected. It has an annual reproductive cycle, spawns from October to November, and has typical larval development (Chen

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and Chen, 1993). In addition, it has intestinal diverticular sand grains that function as weight belts (Chen and Chen, 1994; Mooi and Chen, 1996). Mooi (1990) has suggested that *Sinaechinocyamus* is a highly derived, extremely miniaturized progenetic sand dollar that closely resembles juveniles of *Scaphechinus* in its patterns of oral and aboral plates, fossil record, and geographic distribution. *Scaphechinus mirabilis* is distributed in the waters surrounding Japan, and its maximum size may be 88 mm in transverse diameter (Nisiyama, 1968).

To reveal the mechanisms of miniaturization in *S. mai*, some ontogenetic data, including age, size, growth rate, developmental stages, developmental rate, and mature age, were collected. Generally, age is estimated by growth zones, or rings (Pearse and Pearse, 1975; Gage, 1991), but growth zones are not easily observed in *S. mai*. Thus, the age and growth rate were estimated according to the population dynamics, size at maturity, and annual reproductive cycle. Developmental characteristics include the separation of interambulacral plates on the oral surface, and an increase in the number of tube-foot pore-pairs in the petaloids. Therefore, the developmental stages of oral plates and aboral plates were determined as the number of discontinuous interambulacral plates and the number of tube-foot pore-pairs, respectively. The results reveal that the reduction of growth rate in *S. mai* is a more important mechanism for miniaturization than is precocious cessation.

Materials and Methods

Population dynamics and maturity

From October 1994 to March 1996, individuals of *Sinaechinocyamus mai* were periodically sieved with a 0.5-mm-mesh screen from sand flats in the low tide zone of Tunghsiao (120° 39′ 45″ E; 24° 29′ 21″N), Miaoli, western Taiwan. The sizes of individuals collected were measured as the longest length through the anus.

Spawning was induced in individuals collected during the spawning season (October 1994 and 1995) by injecting 0.1 ml 0.5 *M* KCl into the coelom through the anus. Eggs were then fertilized artificially, and cleavage was verified with a Wild M3Z dissecting microscope. In addition, the gonads of individuals that did not spawn after injection with KCl were removed to determine their maturity. The gonads were fixed in 10% neutral formalin overnight, and then transferred to 70% ethanol, dissected by removing the oral half of the test, and examined with a dissecting microscope. The maturity of the gonads was determined using the methods of Chen and Chen (1993).

Growth rate analysis

According to Chen and Chen (1993), the spawning period of *S. mai* is from late October to early November, so a spawning date of 1 November was used in estimating age. The growth curve was estimated by regression lines from each individual's size and its estimated age (month). The growth rate of *S. mai* was compared with that of *Scaphechinus mirabilis* as reported by Brykov and Parasyna (1979).

Development of oral plates

Tests were collected from the seashore in western Taiwan (including Hsinchu, Tainan, and Tunghsiao). The outlines of oral plates were drawn from tests placed under a Wild M3Z dissecting microscope equipped with a Wild TYP 308700 drawing tube. The visibility of the plates was increased by adjusting the intensity and incidence of the light. Tests less than 3 mm in length could not be studied under the dissecting microscope; therefore they were dried and coated, and then examined with a scanning electron microscope.

The developmental stage of the oral plates was determined by counting the discontinuous interambulacral plates. Each distinctly separate interambulacral plate was counted as 1 point. Plates in the process of separating (*i.e.*, not easy to distinguish whether separated or not) were counted as 0.5 point. Each sand dollar has 10 interambulacral plates, so the score for a test was derived by totaling the number of discontinuous plates among the 10.

Development of aboral plates

To trace the developmental characteristics of aboral plates, individuals were first treated with house bleach to remove the spines. The numbers of gonadal pores and tube-foot pore-pairs were then counted with the aid of a dissecting microscope.

The oral and aboral developmental stages of *Scaphechinus mirabilis* were based on descriptions in other papers (Nishiyama, 1968; Mooi, 1990) and on specimens collected from Japan by Dr. M. Komatsu and Y. Kano. Actual-size sketches of *Scaphechinus mirabilis* were traced in pencil on thin paper covering the tests.

Results

Size-frequency distribution

Living sand dollars were collected at Tunghsiao nine times from October 1994 to March 1996. With the exception of the February 1995 collection containing only 5 individuals, the size distributions are shown in Figure 1. Two size groups clearly existed in the sand dollars gathered in August and October 1995, while the other collections contained a few larger individuals (Fig. 1). One sand dollar collected in October 1994 was 10.9 mm in length.

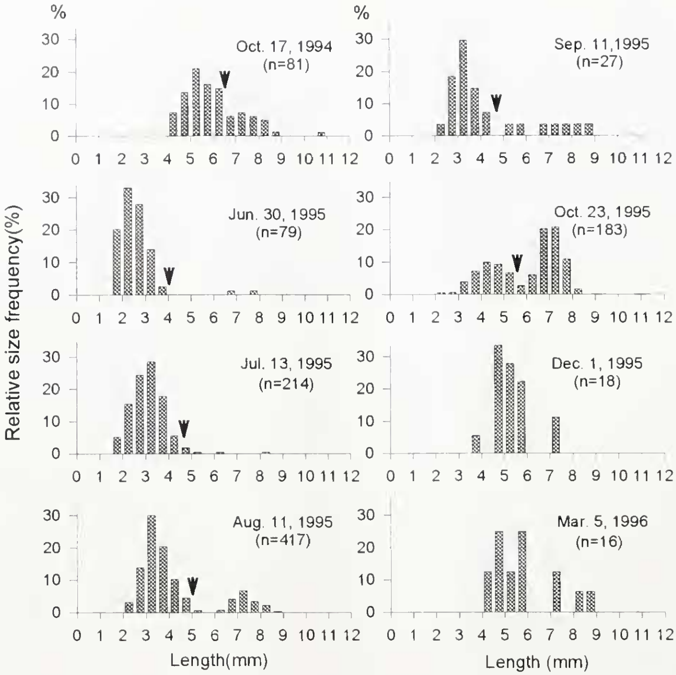

Figure 1. Size frequency distribution of *Sinaechinocyamus mai* from October 1994 to March 1996. Arrows indicate the division between two size groups on each graph except the last two, in which the number of specimens was not sufficient for groups to be discerned.

Maturity of gametes

Only large-sized individuals were able to spawn in response to injection of KCl solution during the spawning season. The 33 male and 32 female sand dollars examined during the spawning season showed no significant sex-related difference in size distribution. Individuals with mature oocytes or sperm were between 5.0 and 6.0 mm in length (Fig. 2).

The growth curve and comparison with Scaphechinus mirabilis

Since the two size groups of *S. mai* were distinct in degree of maturity, the groups were considered to represent different ages: specimens belonging to the small-size group were 1 year younger than their larger counterparts. The extra-large individual (10.9 mm in length) was considered to be a year older than the members of the large-size group.

This sand dollar spawns annually, therefore the 1995 small-size and large-size groups were assumed to be different age groups spawned in 1994 and 1993, respectively. Consequently, the growth rates of the first year and the second year were estimated from the age (in

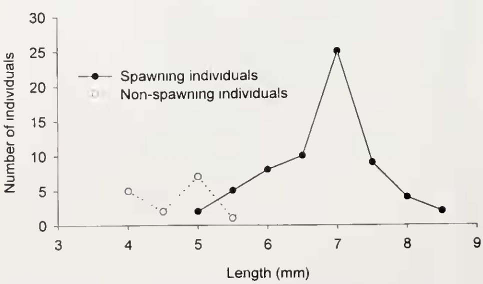

Figure 2. Size and maturity. Only large individuals are mature in spawning season.

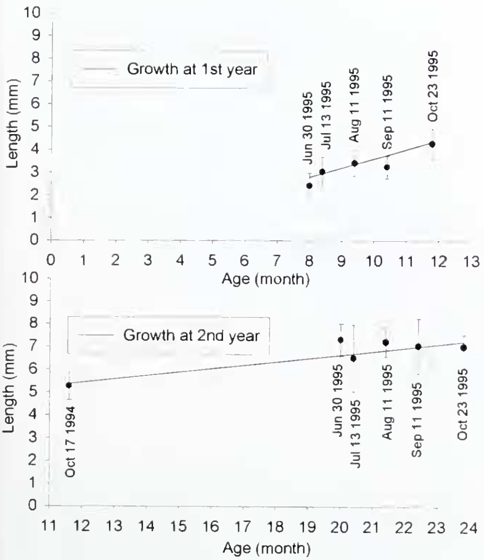

Figure 3. Linear regression between body length and age. Mean and standard error are shown for each age group.

months) and the length (in millimeters) of small-size and large-size groups, respectively. The regression equations are as follows:

$$Y1 = -0.457 + 0.404\,(X1),$$

$$(r = 0.307,\ t = 8.644,\ n = 720,\ P < 0.01)$$

$$Y2 = 3.616 + 0.150\,(X2),$$

$$(r = 0.544,\ t = 10.393,\ n = 259,\ P < 0.01)$$

From these two equations, the annual growth rate of the first year was estimated to be 4.4 mm/y ($Y1(12)$), and that of the second year was estimated to be 1.8 mm/y ($Y2(24) - Y2(12)$) (Fig. 3).

The annual growth rates of *Scaphechinus mirabilis* in the first and second years are 23.4 and 9.2 mm/y, respectively (Brykov and Parasyna, 1979). Therefore, the growth rates of that species in the first and second years are respectively 5.3 and 5.1 times faster than those of *S. mai*, and *Scaphechinus mirabilis* would be about 5.2 times larger than *S. mai* at the same age. Thus, the developmental rate of these two sand dollars should be compared by taking the actual-size pattern of *S. mai* and contrasting it with a reduced-size (to 19%) pattern of *Scaphechinus mirabilis*.

Developmental stages and rate of oral plates

Interambulacral plates separate in a specific order: posterior; posterior-right and posterior-left (whether posterior-right occurs second and posterior-left third or vice versa is still unclear); anterior-right; and anterior-left (Fig. 4A–F). Examination of the discontinuous interambulacral plates revealed that *S. mai* had a pattern different from that of *Scaphechinus mirabilis* when analysis was based on actual size. However, the full-size *S. mai* pattern was similar to the reduced-size *Scaphechinus mirabilis* pattern (Fig. 5). This indicates that development of oral plates is age-dependent.

One sample that was 8.3 mm in length had clear outlines of two rings on each plate (Fig. 6A, B). A model was constructed by assembling plates equivalent in size to the smaller of the rings on each plate of the 8-mm test. The resulting composite was similar to that of an individual 5.5 mm in length (Fig. 6B, C). The space between the rings indicated that the growth rates of plates in ambulacra were faster than in interambulacra. This allometric growth is identified as the process that causes separation of the interambulacral plates.

Developmental stages and rate of aboral plates

The number of tube-foot pore-pairs increased linearly with the increase in body length, from 8 pairs for a 1.5-mm body to 124 pairs for a 9.8-mm body. The number increased continuously and was not interrupted by the development of gonads and the formation of gonadal pores (Fig. 7). The increasing number of tube-foot pore-pairs showed that *S. mai* had a pattern similar to that of *Scaphechinus mirabilis* based on actual size, but dissim-

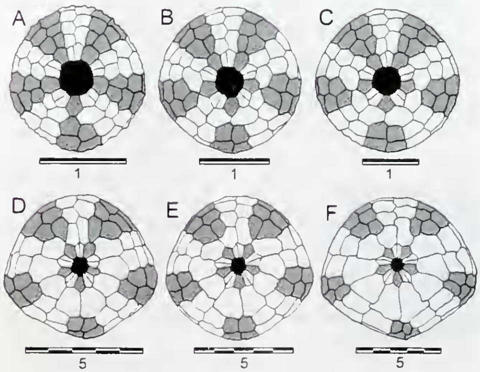

Figure 4. Developmental stage of oral plates. The interambulacral plates separate as size increases. Interambulacral plates are shaded; all scale bars are in millimeters.

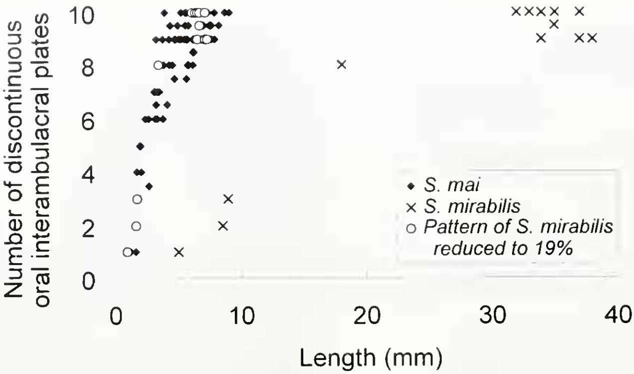

Figure 5. Developmental stages and developmental rate of oral plates.

ilar to the reduced *Scaphechinus mirabilis* pattern. That is to say, the increase in number of pore-pairs in *S. mai* was reduced by the same ratio as the reduction in growth rate.

Discussion

This study yielded several important results. First, the growth rates of *S. mai* are extremely slow: 4.4 mm/y in the first year and 1.8 mm/y in the second year. In *Scaphechinus mirabilis*, the corresponding rates are 23.4 mm/y and 9.2 mm/y (Brykov and Parasyna, 1979). The growth rate of *S. mai* is thus about five times lower than that of *Scaphechinus mirabilis*. Second, on the basis of size, the developmental stages of the oral plates (the increase in the number of discontinuous interambulacral plates) in *S. mai* is far "faster" than in *Scaphechinus mirabilis*, but the developmental stages of the aboral plates (the increase in the number of tube-foot pore-pairs) are almost the same in both sand dollars. However, age should be the basis for the comparison of developmental rates. When so compared, the developmental rates of the oral plates are the same in both sand dollars, but the developmental rate of the aboral plates of *S. mai* is only 19% of that found in *Scaphechinus mirabilis*. Consequently, *S. mai* closely resembles juveniles of *Scaphechinus mirabilis*. Third, individuals of *S. mai* mature sexually (*i.e.*, have the ability to spawn) at the age of 2 years. Echinoids are typically 30% to 40% of their maximum size at first reproduction (Pearse and Cameron, 1991). Because the maximum size of *Scaphechinus mirabilis* is about 88 mm in length, the size of this species at first spawning is estimated to be 26.4–36.2 mm, which is larger than its 1-year-old size (23.4 mm), but includes its 2-year-old size (32.6 mm). These results suggest that the age of first spawning for *Scaphechinus mirabilis* would be between 2 and 3 years, close to the 2-year figure for *S. mai*.

As the growth rate of *S. mai* is five times slower than that of *Scaphechinus mirabilis*, there is no doubt that it has a tremendous effect on size. There is probably no difference between the ages of sexual maturity in *S. mai* and *Scaphechinus*. By inference, then, the mechanism for the miniaturization of *S. mai* is a reduction in growth rate. Thus, Mooi's suggestion of progenesis (1990) must be modified to neoteny. Since aboral and oral morphological characteristics have differences according to size, this miniaturized evolution is a dissociated heterochrony and the descendant, *S. mai*, is not a microcosm of its ancestor, *Scaphechinus mirabilis*.

Tube feet must attain a certain size in order to perform their normal function (Lawrence, 1987). Thus, the number of tube-foot pore-pairs located on the aboral plate is obviously dependent on the size of the plate. In *S. mai*, growth reduction keeps the aboral plates small, so only a few tube-foot pore-pairs can develop. Having established

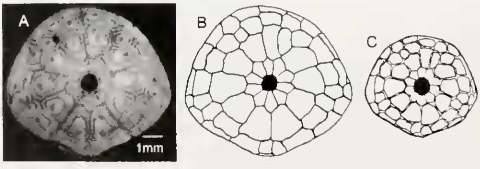

Figure 6. Original pattern of growth rings and the reassembly of first rings. The space between rings demonstrates that the growth rate in ambulacral plates is faster than in interambulacral plates. This allometric growth produced the separation of the interambulacral plates.

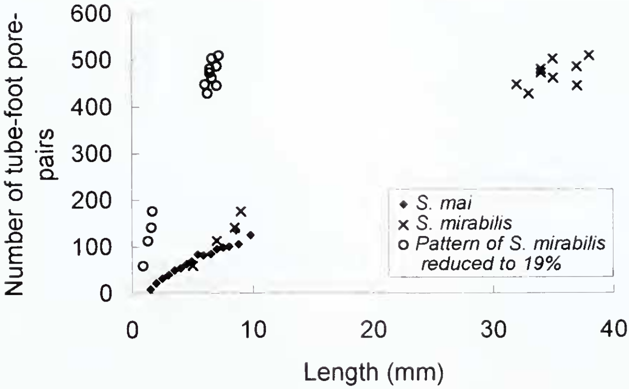

Figure 7. Developmental stage and developmental rate of aboral plates.

that this is a size-dependent characteristic, we propose that other characteristics responsible for the similarity of adult *S. mai* to juvenile *Scaphechinus mirabilis* may also be dependent on size. These would include aboral periprocts, simple internal partitions, and food grooves (listed by Mooi, 1990).

The development of oral plates in terms of the number of discontinuous interambulacral plates is a continuous process of plate rearrangement, and it is somewhat independent of size. Changes in onset, offset, and growth rate in different oral plates may cause different consequences; thus, the development of discontinuous plates becomes very variable in sand dollars (as in *Echinarachnius parma;* see Lohavanijaya and Swan, 1965). Comparisons of this characteristic should be limited to species that are closely related, like the ones in this study.

Sinaechinocyamus has existed in Taiwan since the Pliocene Epoch (Wang, 1984); *Scaphechinus* was recorded in the Pliocene Epoch in both Taiwan (Hayasaka and Morishita, 1947) and Japan (Mooi, 1989), but is extant in Japan only. Since both genera occurred simultaneously during the Pliocene, the initiation of speciation in *Sinaechinocyamus* is difficult to explain, but the success of miniaturization may be related to the presence of sand grains in the diverticula for position maintenance (Chen and Chen, 1994).

Mean global temperature dropped from the Miocene Epoch to the Quaternary Ice Age. Afterwards, the trend reversed, and global temperature reached its current state (Skelton, 1993). On the basis of water temperature changes and the geographical and stratigraphic occurrence of these two genera, we propose that *Sinaechinocyamus* might have evolved from a local, marginal population of *Scaphechinus* that extended to Taiwan from the waters of Japan during the early Pliocene Epoch when the sea temperature was low. When water temperature rose, only miniaturized *Sinaechinocyamus* were able to continue their existence down to the present time in Taiwan. We hypothesize that the selective pressure on *Scaphechinus* was due to the warming sea temperature, which would increase metabolic rates, exhausting the materials and energy needed for maintenance and growth and leading to the disappearance of *Scaphechinus* from Taiwan. Conversely, a slow grower, even with the same metabolic rate, might have been able to tolerate the disorder caused by a warming of the water temperature. Thus, we speculate that the advantage of the small size of *Sinaechinocyamus* is related to the slow growth rate, which passively allows for existence in warmer waters.

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